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Green Synthesis, Characterization and Biological Assessment of Silver Nanoparticles by using *Artemisia carvifolia*

by

Naqoosh Zahra

A thesis submitted in partial fulfillment for the degree of Master of Science

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Dedicated to Almighty ALLAH and the Holy Prophet Muhammad (P.B.U.H)
and My Loving Family.

CAPITAL UNIVERSITY OF SCIENCE & TECHNOLOGY
ISLAMABAD

CERTIFICATE OF APPROVAL

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Abstract

In recent and modern studies of science, the most burning field in science for the researchers is nanotechnology. Nanotechnology administers the nanoparticles of size ranging from 1-100 nm. There is a tremendous use of nanoparticles due to their miniature size, orientation and substantial properties. By means of different physical, chemical, and biological approaches, these petite particles can easily be prepared. However, the biological approach is majorly an emerging technique for preparation of nanoparticles, for the reason that this method is much less complicated in comparison with other approaches because it is much less time consuming, and more importantly eco-friendly. Green synthesis of nanoparticles was carried out by mixing an aqueous solution of leaf extract of *Artemisia carvifolia* and silver nitrate $AgNO_3$ solution. Silver has particular significance to this procedure due to its evocative chemical and physical properties. Plant extract and metal ion solutions were mixed together in a fixed ratio and after that color change in the solution was observed, this change in color signified that nanoparticles are formed. The nanoparticles were characterized by using instruments that includes UV-vis spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscope (SEM). The optical properties of silver nano particles using UV- vis spectrophotometer has shown the absorbance peak at the 450 nm. Through SEM analysis, the polyhedral and leaf shaped silver nanoparticles were found and the size of silver nanoparticles range between 80-120 nm, whereas FTIR analysis has provided the information about the bioactive compounds that are aldehydes, ketones, alcohols and amines of extitia carvifolia were rsponsible for the stabilization and capping of silver nanoparticles. To validate the activities of the synthesized nanoparticles, different biological assays were analyzed that comprises antibacterial, antioxidant and cytotoxic assays. Antibacterial activities of silver nanoparticles has shown inhibition against both the Gram-negative strain as well as Gram- positive strain. The antioxidant activities of silver nanoparticles has shown a high scavenging in total antioxidant capacity as compared to total reducing power and DPPH free radical scavenging assay. The Cytotoxic assay has shown the LD_{50} value at 19.06 μg/ml, demonstrating a strong cytotoxic potential.

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Abbreviations

Ag-NPs	Silver Nanoparticles
A.carvifolia	*Artemisia carvifolia*
ACAgNPs	*Artemisia carvifolia* Silver Nanoparticles
UV-vis	Ultra Violet Visible Spectroscopy
SEM	Scaning Electron Microscope
FTIR	Fourier Transform Infra-Red Spectroscope
$AgNO_3$	Silver Nitrate
SPR	Surface Plasmon Resonance
PBS	Phosphate Buffered Saline
nm	Nano-meter
rpm	Rotation per minute
μL	Micro litter
μg	Micro gram
mL	Mili liter
KBr	Potassium Bromide
min	Minutes
mV	Mili volt
Hrs	Hours
mM	Mili molar
ppm	Parts Per Million

Chapter 1

Introduction

1.1 Background

Our environment undergoes a massive collapse, due to rapid urbanization and industrialization, plus a large number of chemicals, gases or hazardous and superfluous materials are unconstrained, so at this point, we necessitate to be acquainted with the secrets residing in nature and its natural goods that is directed towards the advancements required in the processes of synthesizing the nanoparticles. For biological molecules, nanotechnological applications are very fitting, thanks to their exclusive properties. In the synthesizing process of metal nanoparticles, the biological molecules go through an extremely illicit assembly to make them suitable, which have proved to be reliable and reverential for the environment [1]. The course of action for the synthesis of nanoparticles that are metal and semiconductor in nature have an immense zone of research that is entitled to its impending applications that are instigated to develop the novel and innovative technologies [2]. One of the imminent research areas in the prevailing material science arena is a field of nanotechnology. Nanoparticles have demonstrated a fully brand newfangled or enhanced properties, like their size dimension, dissemination, distribution, and morphology of particles. Unique and the unusual applications and uses of nano-materials plus nanoparticles are swiftly emerging in different domains [3].

Metal nanoparticles consist of a highly particular and particular surface area and also contains an elevated fraction of surface molecules/atoms. Keeping in view, the remarkable physicochemical qualities of the nanoparticles, comprising optical properties, synergist/catalytic actions, magnetic properties, antibacterial properties, and electronic properties [4] [5] [6] [7] they are gaining a peak interest among scientists because of these novel characteristics, . In current and modern material sciences, a vital topic of research over the time period of past few years is focused and committed to the synthesis of metal nanoparticles. Nano-crystalline silver particles have been discovered to have huge and humongous applications in the different turfs of highly sensitivity biomolecular recognition, diagnostics, catalysis, microelectronics, therapeutics, moreover for antimicrobial activities. In any case, there is still a requirement for the economically, financially, commercially viable and feasible, on top of them is the environmentally clean routes to incorporate silver nanoparticles. Silver ions are remarkably recognized for retaining an inhibitory influence en-routed for various microorganisms and bacterial strains that are frequently available in the industrial and medical procedures [8]. In the medicines arena, silver nanoparticles and the silver itself holds sufficient enough applications comprising products of skin creams and ointments that encloses silver to preclude the burns and wounds infection [9], silver-impregnated polymers are used to prepare medical devices and implants [10]. Currently in the textile industry, sporting equipment prepared with the silver-embedded fabrics [11].

Nanoparticles can be incorporated utilizing different methodologies including compound physical, chemical and organically biological. Albeit chemically synthetic technique for the synthesis requires a quite brief timeframe for the development of an expansive amount of nanoparticles, this strategy requires the capping operators for size adjustment and stabilization of nanoparticles. Synthetic chemical concoctions utilized for the nanoparticles amalgamation, maintenance and stabilization are harmful, toxic and prompt towards the contaminated and non-ecofriendly by-products. The requirements needed to produce ecological non-lethal/non-toxic engineered protocols for the nanoparticles amalgamation had prompted the fashioning interest for the biological methodologies which are free from the deployment

of any dangerous and toxic compounds as by-products. Along these lines, there is an escalating interest in "green nanotechnology" [12]. Many biological methodologies for both of the intracellular and extracellular nanoparticles amalgamation have been ensured and also been developed by the utilization of microorganisms including plants, fungi, and bacteria [13] [14].

Plants provide a much superior edge to nanoparticles synthesis as they are free from any poisonous toxic chemically synthetic substances and also bounce off characteristically natural agents for capping. Besides, utilization of plant extricates additionally diminishes the cost of isolation and culture media of micro-organisms, upgrading the cost competitive possibility over the synthesis of nanoparticles by microorganisms [12]. At times the nanoparticles synthesis by consuming different plants plus their concentrates are highly invaluable over the other natural biological synthesis procedures which take account of the specific complex techniques for the maintenance of microbial societies [15] [16]. Such numerous investigations involving microorganisms in the synthesis of nanoparticles have been established, for example, utilizing fungi like *Penicillium* sp [17] for the synthesis of different metal nanoparticles, also *Fusarium oxysporum* [18], and utilizing a few microbes such as *Bacillus subtilis* etc [19] [20]. However, the blend of nanoparticles with the floral extracts is the most embraced technique for green, economical and environmentally friendly formation of nanoparticles, and moreover, has an amazing auspicious point that the floral regions are much widely distributed, effectively comprehensible, considerably more dependable to deal with and most importantly they are fountainhead of metabolites that are necessary to stabilize and capped the nanoparticles [21]. There has been a few trials performed for silver nanoparticles synthesis by utilizing therapeutic plants, for example, *Sorghum bicolour, Oryza sativa, Saccharum officinarum, Helianthus annus, Zea mays, Medicago sativa (Alfalfa), Cinamomum camphora, Basella alba, Magnolia kobus, Capsicum annuum, Geranium sp. and Aloe vera* in the field of pharmaceutical applications and biological ventures. In addition, silver nanoparticles green synthesis by means of *Eucalyptus hybridawas* methanolic extract was like-wisely be examined [22].

Nowadays, the synthesis of silver nanoparticles carried out from the natural resources and their substances like *Neem (Azadirachta indica), green tea (Camellia sinensis), leguminous shrub (Sesbania drummondii), lemongrass* leaves extract, natural rubber, different broth leaf, starch and *Aloe vera* and, so on [23]. As for the microorganisms, the silver nanoparticles become connected to the cell wall, accordingly irritating permeability of cell wall and their cellular respiration. The nanoparticles might infiltrate somewhere profoundly within the cell wall, hence instigating the cellular damage by interfacing with the compounds of sulfur and phosphorus, for example, protein and DNA present in the bounds of the cell. Bacteriocidal properties of silver nanoparticles are because of heavy influx of the silver ions from metallic particles, that are acquainted with the antimicrobial action [24]. Correspondingly, the intensity levels of the impacts of antibacterial actions correlates with the size of nanoparticles. Thus indicating that smaller and minor particles exhibits higher antibacterial exercises, because these petite particles are loaded with the abundant and even silver mass substance. The extent for the clinical utilization of the nanoparticle, microorganisms including fungi, bacteria, diatoms, and yeast producing inorganic materials are done with the natural biological formation each from intracellularly or extracellularly, have crafted these nanoparticles to be more biocompatible [25].

Besides, the utilization of plant extracts has lessened the expenditure of microorganism's disengagement and their media culture by upgrading the cost-focused practicality over the nanoparticles formation by the usage of microorganisms [26]. Nanotechnology is an interdisciplinary maneuver in the arena of biochemical applications that has been focused on the nanoparticles synthesis, having an enhanced antioxidant and antimicrobial properties against the degenerative disease, cancer and tumors [27].

Current research and enquiries trends in the both natural and manufactured antioxidant agents had led to the identification and screening proofs for the new anti-oxidants from floral sources. Synthetic antioxidants are accounted for, to have different properties, for example, anticarcinogenicity, antiallergencity, anti-aging activity and hostile to mutagenicity [28]. Plant extract antioxidant abilities

are because of the redox capability of phytochemicals [29], which can assume a critical part in extinguishing singlet and triplet oxygen, peroxides break down or the free radicals carnage. In this manner, its to be expected that the enhanced nanoparticles antioxidant activity is maybe because of the particular adsorption of antioxidant material from the concentrate onto the surface of nanoparticles. Distinctive parameters like size particle, surface zone, and surface reactivity decides the harmfulness and toxicity of the nanoparticles in floral extricates.

The cytotoxicity impacts of silver nanoparticles on the cancer cells was reported by Jacob et al. [30]. Because of the microbial resistance, metallic nanoparticles have increased more consideration from analysts [31] and among other different metals, silver is of more significance because of its crucial part as the antimicrobial specialist [32].

The reliance of human beings on the plant kingdom for nourishment, food, grain, fodder, fuel, medicinal, and restorative reasons are as old as the human's living on this planet. The plant kingdom is a reservoir of profitable restorative medicinal flora and the utilization of these plants to fix different maladies/diseases have been dated back to 1500 BC [33]. Undeniably, the knowledge and information of natural herbal medicines were identified by a network community, rehearsed, practiced, heir-loomed and beneficiary lingered to the progressive ages and generations [34]. Albeit, a few synthetically engineered drugs are accessible to treat different infections, diseases, and disorders, but they are at the cost of many side-effects [35]. Then again, there is an expanding interest for the herbal meds because they are safe, protected, powerful, effective, prudent, economical, eco-accommodating and free from pernicious impacts. Herbs are a huge wellspring of optional secondary metabolites which ensure them against micro-organisms, animals, and birds, and also draw out the plant pollinators as [36]. A few secondary metabolites have turned out to be exceptionally helpful for the generation of pharmaceutical medications for human medicinal services. A broad investigation of the phytochemistry of the family *Artemisia* has prompted the recognizable proof of different biochemically dynamic secondary metabolites including fundamental oils, flavonoids, esters, terpenes, and unsaturated fatty acids [37].

Artemisia is a broad and widespread Genus which includes excessively of about 400 species (474) and is venerated as 'Worm wood', 'Sagebrush' 'Mug word', or 'Tarragon' [37] [38]. The word '*Artemisia*' originates from the old Greek word: 'Artemis'=The Goddess (the Greek Queen *a*) and 'asinthium'=Unenjoyable or without sweetness. The word 'Wormwood' is affected by the conventional use as a solution for intestinal worms. Most of the *Artemisia*s are bianual, perennial, annual herbaceous ornamental, aromatic plant or shrubs and medicinal. Their appearance is in silver green, dark green or blue-green in color, and have a sharp smell and unpleasant taste because of the presence of sesquiterpene and terpenoids lactones [39].

Of all the significant uses of *Artemisia* species, the most important one is that it is utilized as a liniment that is an intense pain reliever [40] [41]. The monoterpenoids in the plant cooperate with transient receptor potential cation channels to assuage the pain and ache. The plant as well contains sesquiterpenes that might be engaged to help with the discomfort. The liniment is much more powerful and effective than opioid sedates and is considerably more secure. A little volume of the liniment is connected where it is required. Within the time span of 20 min, the torment and pain subside down even agony from broken bones, joint inflammation, sprains, arthritis, and strains [42].

Artemisia species are widely utilized as a part of traditional and conventional medicinal drugs everywhere throughout the world with various and surely understood therapeutic and helpful applications (stomach ache, parasitism, diarrhea, bronchial and intestinal infections, pimples, angina, wounds, coughs, and colds) [43] [44]. Genus *a* is an etravagant wellspring of terpenoids and other secondary plant items having usage as a part of pharmaceuticals and perfumery [45]. As indicated by literature, more than 260 *Artemisia*s have bee examined to uncover that they contain numerous classes of secondary metabolites including glycosides, terpenoids, flavonoids, sterols, coumarin, and polyacetylene [46]. Among the secondary metabolites of the *Artemisia*, sesquilactones ad flavonoids

are of high therapeutic and restorative significance. They exhibit antitumor, anti-inflammatory, antioxidant, antimicrobial, antispasmodic, antimalarial, insecticidal, and antifungal activities [44] as well as they increase immunity and decrease the risk of atherosclerosis, arthritis, and gastrointestinal disorders [47] [48] [49]. That's why this plant was selected to form the nanoparticles so that it will be helpful in treating diseases at the exact minute levels.

1.2 Aims

With the passage of time we are facing the problem of drug resistance in a number of diseases. Nanoparticles have been successful in enhancing the therapeutic effects of drugs and reducing the side effects. We need to develop new effective drugs from natural sources. For that purpose the present study is aimed to identify, synthesis of silver nanoparticles and their characterization using UV-Vis, SEM, and FTIR, and also to examine the antimicrobial, antioxidant and cytotoxic activities of silver nanoparticles using medicinal plant *Artemisia carvifolia*. The main purpose of this study is to generate stabilized nanoparticles with much less effort and economic value and to find out that silver nanoparticles have enhanced biological activities as compared to its plant extract to fight against various therapeutic issues.

1.3 Objectives

This study entails the following objectives:

- Synthesis of silver nanoparticles by using the medicinal plant *Artemisia carvifolia*.
- Characterization of the biosynthesized silver nanoparticles by "UV- spectrophotometer", and to identify the shape and size of silver nanoparticles by

"SEM" (Scanning Electron Microscopy) analysis. "FTIR" (Fourier Transform Infrared Spectroscopy) analysis for the identification of bioactive compounds in plant extract, utilized in the synthesis of silver nanoparticles.

- To evaluate the antibacterial activity of the silver nanoparticles against both Gram-negative and Gram-positive bacterial strains.
- To assess the capability of nanoparticles against free radicals by means of the antioxidant assay.
- To check the apoptotic abilities of nanoparticles using brine shrimp cytotoxic assay.

Chapter 2

Literature Review

In modern research, nanotechnology is one of the most important fields which deals with synthesis, manipulation, and designing of particles and modifying their structures from 1-100 nm approx. depending upon its dimension. Similarly, technology is remarkably growing day by day which has opened novel applied and fundamental frontiers which also includes nanoscale synthesis of different materials and their utilization or exploration according to their properties i.e. optoelectronic and physicochemical. Nanotechnology is rapidly gaining importance in various sectors such as food and feed, cosmetics, healthcare, mechanics, environmental health, optics, biomedical sciences, space industries, electronics, chemical industries, optoelectronics, drug-gene delivery, energy science, single electron transistors, light emitters, nonlinear optical devices catalysis, and photoelectrochemical applications [50] [51]. It is, therefore, seen as a potential solution to various environmental and technological challenges in fields related to medicine, solar energy conversions, the process of catalysis and water management.

Green synthesis methods are accompanied by nanomaterials, in order to control the hazardous effects of waste on a global level. Its demand is also increasing with the passage of time. Thus, nanotechnology is potentially changing the methods of synthesis of various materials and fabrication of devices. Nanoscales blocks can incorporate into functional assemblies and several multifunctional devices by using "bottom-up approach". Currently, researchers are interested in the synthesis

of nanomaterials due to their properties such as magnetic, optoelectronic and mechanical, which differs from other materials [52].

2.1 Nanoparticles

Nanoparticles are particles which vary from 1 nm – 100 nm in size in at least one dimension out of three. Due to the size of this particular range, the properties (biological, physical and chemical) of nanoparticles changes at the atomic/ molecular level, this makes these nanoparticles unique. Nanoparticles can be synthesized by using a material with diverse chemical nature such as metals and their oxides, silicates, non-oxide ceramics, carbon, organics, biomolecules, and polymers. Morphologically, nanoparticles are found in distinctive shapes such as spheres, cylinders, tubes, and platelets etc. However, these are specifically designed and modified after considering the applications they are being used for. The diversity of nanoparticles depending upon their shape, chemical nature, morphology, medium, surface modification and state of dispersion is shown in Figure 1. These are the features that make nanoparticles an important part of science fields nowadays.

2.2 Categories of Nanoparticles

Nanoparticles can be divided into two groups depending upon their organic (carbon nanoparticles) and inorganic nature (magnetic, noble metal including silver and gold, and semiconductor nanoparticles like zinc oxide and titanium oxide). However, researchers have shown more interest in inorganic nanoparticles due to their versatile functions. Gold and silver are on top of the list. Inorganic particles are also proved to have high potential to be used as tools for medical treatments and imaging. Inorganic nonmaterial is also castoff for cellular delivery as they have resourceful features like rich functionality, wide accessibility, the aptitude for targeted drug delivery, good compatibility and meticulous drugs release [53].

2.3 Silver Nanoparticles

Silver nanoparticles pose unique properties depending upon their size and morphology i.e. electrical, optical and magnetic) and due to this particular reason, they are high in demand. They can also be incorporated into antimicrobial applications, biosensor materials, cryogenic superconducting, composite fibers, materials, cosmetic products and electronic components. Several methods i.e. physical and chemicals have been used for the synthesis and stabilization of silver nanoparticles [54] [55].

Number of well-known processes such as chemical reduction by using different inorganic and organic reducing agents, physicochemical reduction, radiolysis, and electrochemical techniques are broadly expended for synthesizing the silver nanoparticles. Recently, synthesization of nanoparticles is one of the most remarkable scientific subjects and new technologies and methodologies are being introduced in this field day by day. There is also growing attention towards the production of nanoparticles by using environment-friendly methods such as green chemistry. Advances in the Green synthesis methodologies have an advantage over the other conventional methods as it includes tollens, polysaccharides, mixed-valence polyoxometalates, irradiation, and biological method which doesn't involve chemicals connected with the environmental toxicity. This chapter represents the impressions of the synthesis of silver nanoparticles by concerning different physical, chemical, and the green synthesis tactics. This part consequently reflects both the present state as well as future prospects, particularly the possibilities and constraints of the previously mentioned strategies for the industry ventures. Additionally, utilization of silver nanoparticles and their integration into other materials have likewise been conferred.

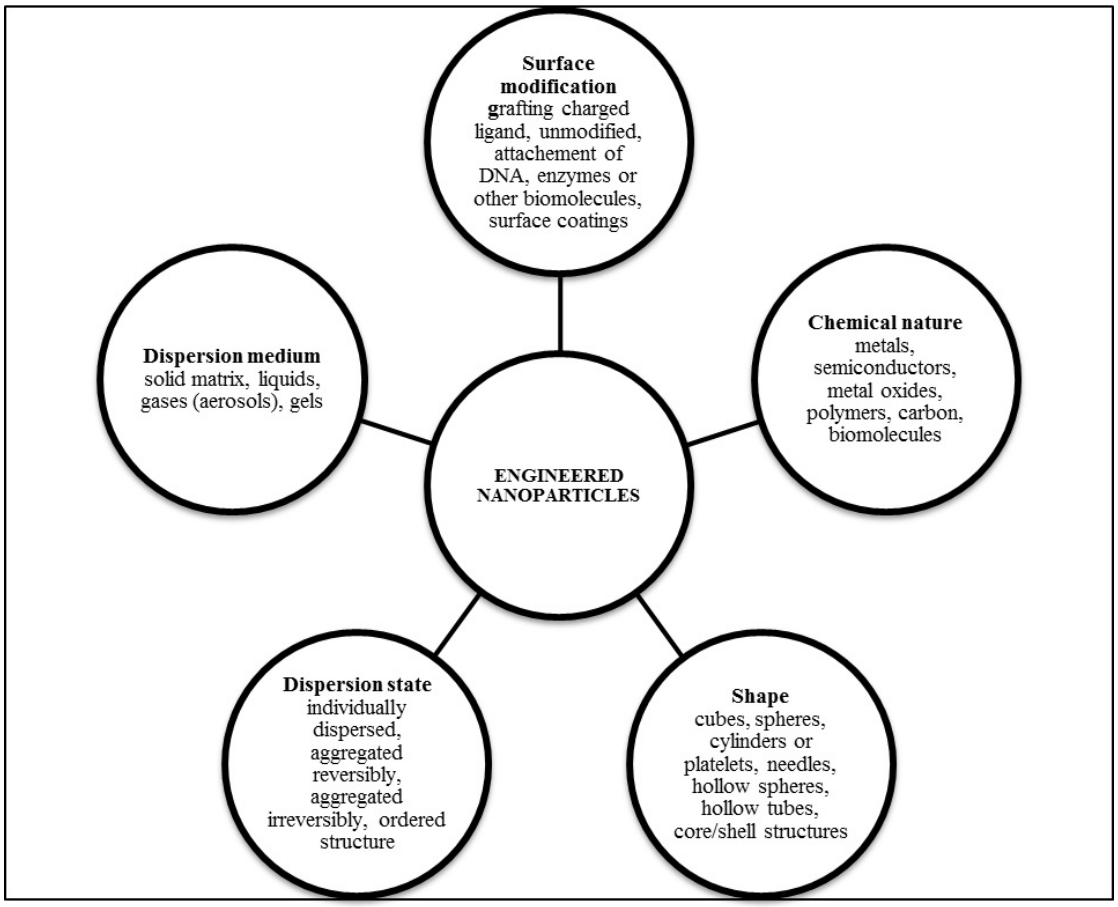

FIGURE 2.1: Variety of engineered metal and non-metal nanoparticles. The similar substances are capable of generating a wide-ranging variety of nanoparticles [56].

2.4 Methods of Nanoparticles Synthesis

Worldwide, nanoparticles have been made physically and chemically for many years, but recently modifications and improvements involve microorganisms and several biological systems in synthesis and production of metal nanoparticles. (Figure. 2.2)

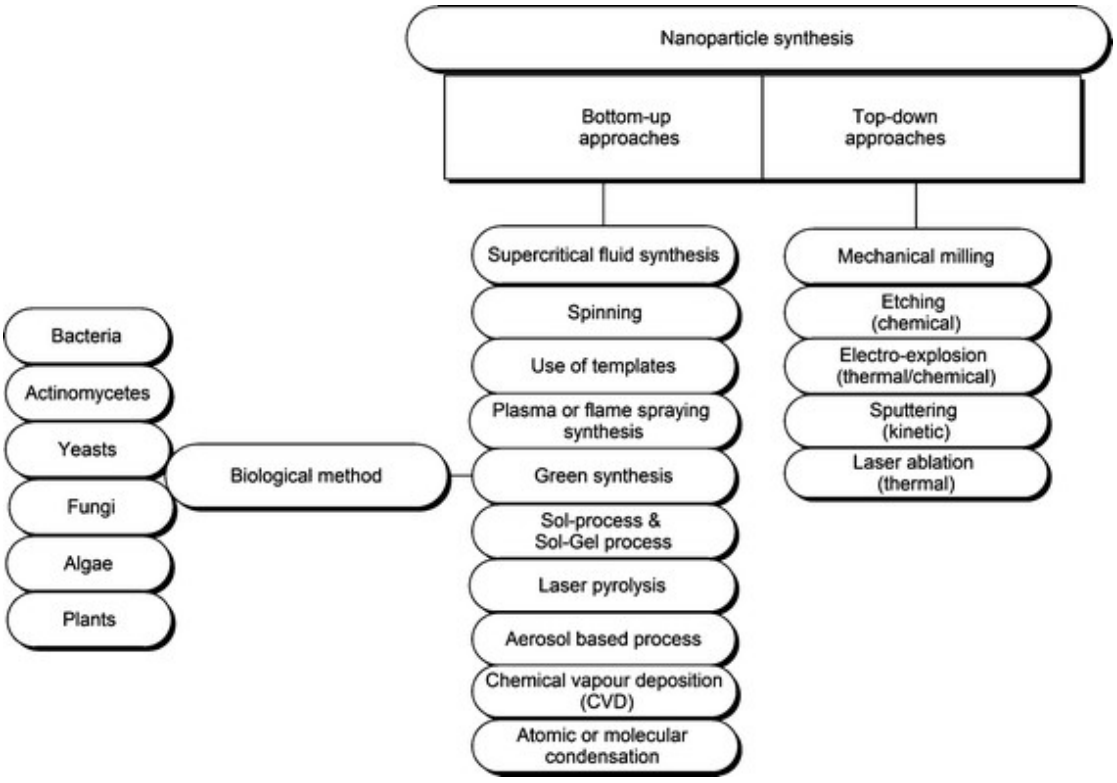

FIGURE 2.2: Important methods used in the nanoparticles synthesis [57].

2.4.1 Physical Approaches

Evaporation-condensation and laser ablations are the most used and important physical approaches. Most of the metallic nanoparticles including silver, lead sulfide gold, cadmium sulfide and fullerene were produced by using the evaporation-condensation method. In physical approaches, there is no possibility of solvent contamination in thin films and there is a uniform distribution of nanoparticles, unlike chemical approaches [58] [59]. Silver particles can be synthesized by using a trifling ceramic heater having the local heating basis [60].

Once these vapors are evaporated, they are allowed to cool at a suitable temperature rapidly; due to the gradient temperature in a surface of the heater is extremely steep as compared to that of the furnace tube. This drop in temperature makes the nanoparticles formation possible at the very small size and in much higher concentration. This system can be useful in long-term experiments on nanoparticles production which can be used for calibration of the device for nanoparticles

measurement and inhalation toxicity studies [60]. Laser ablation can also be used for silver nanoparticles synthesis of the bulk of metallic materials in solution [61] [62] [63] [64] [65].

The efficacy of ablation and the characteristics of nanosilver particles which are synthesized depends upon several factors i.e. the laser impinging wavelength focus on metallic target, laser pulses duration (in pico-, femto-, plus the nanosecond administration), time duration of ablation, the laser fluence, and operative liquid medium, with or without the presence of surfactants [66] [67] [68] [69]. Laser ablation technique is better in comparison with other techniques due to metal colloids production in the absence of solutions with chemical reagents in them. Therefore, pure and uncontaminated metal colloids can be synthesized for various applications by using this system [70].

2.4.2 Chemical Approaches

Chemical reduction is the extremely familiar method for the production of silver nanoparticles by using inorganic and organic reducing agents. In a broad-spectrum, various reducing agents such as ascorbate, sodium citrate, sodium borohydride ($NaBH_4$), polyol process, tollens reagent, elemental hydrogen, poly (ethylene glycol) - block copolymers, and N, N- dimethylformamide (DMF), are used for the reduction of silver ions (Ag+) in non-aqueous or aqueous solutions, and these reducing agents help in the reduction of silver ions (Ag+), which leads to formulate the metallic silver (Ag^O), followed by agglomeration into clusters of oligomers. These clusters of oligomers eventually leads to the formation of metallic colloidal silver nanoparticles [71] [72] [73].

Stabilization of the dispersive nanoparticles during the formulation process of metal nanoparticles and the protection of nanoparticles is possible by using protective agents. Therefore, they are important and help in absorption on the targeted surface and avoid agglomeration [74].

The existence of surfactants including functionalities (e.g., amines, alcohols, acids and thiols) for associations with nano-molecule surfaces can balance out the nano-molecule development, and shield particles from agglomeration, sedimentation, or eluding their surface properties. As of late, controlled size of silver nanoparticles is synthesized by using the Tollens method. In a reformed Tollens system, silver particles are lessened by saccharides within the sight of ammonia alkali, that yields the silver hydrosols (20 - 50 nm), silver nanoparticle films (50 – 200 nm), and the silver nanoparticles of various shapes [75].

2.4.3 Biological Approaches

In the past few years, green chemistry methods have been widely used as they help in natural capping, reduction, capping, and also act as a stabilization agents for the preparations of silver nanoparticles. These particles have turn out to be a major focus among researchers due to their desired size and morphology. Silver nanoparticles can be synthesized via Biological methods without using any toxic, harsh and expensive chemical substances [76] [77].

The bioreduction process of metallic ions which are formed by combinations of biomolecules (e.g., polysaccharides, enzymes/proteins, vitamins, and amino acids) found in extracts obtained from different organisms is environmentally benign, however, it's chemically complex. A number of studies have concluded a successful formulation of the silver nanoparticle by using microorganisms and biological systems [74] [75].

2.4.3.1 Silver Nanoparticles Synthesis by Bacteria

Pseudomonas stutzeri AG259 strain was the first one to be used for synthesizing silver nanoparticles which were isolated from silver mine [78]. Some microorganisms have developed resistance against the metal due to which they can survive metallic ion concentrations. This mechanism involves efflux systems which deal with an alteration in solubility and toxic concentrations via reduction or oxidation,

bioaccumulation, biosorption, precipitation of metals or extracellular complex formation and absence of specific metal transport systems [79]. However, at very high concentrations, these organisms can induce toxicity. Worldwide, the most recognized procedure for the synthesis of silver nanoparticles is in the manifestation of enzyme named nitrate reductase which has a tendency to convert nitrate into nitrite. In *in vitro* amalgamation of silver utilizing microscopic organisms like bacteria, the company of alpha-nicotinamide adenine dinucleotide phosphate lessened frame (NADPH) - dependent nitrate reductase would evacuate the downstream handling advance that is required in different cases [80].

2.4.3.2 Silver Nanoparticles Synthesis by Fungi

Fungi have the ability to produce a large number of nanoparticles as compared to bacteria due to excessive proteins secretion [81]. The process includes subsequent steps: Ag+ ions trapping on fungal surface and reduction process of silver ions via enzymes present in the fungi [82]. The extracellular enzymes i.e. anthraquinones and naphthoquinones are known to expedite the process of reduction. For example, for *F. oxysporum,* it is considered that nanoparticle formulation is based on the NADPH-dependent nitrate reductase and extracellular process of shuttle quinine [83].

However, the exact mechanism is yet to be deciphered, therefore, it is believed that this process is responsible for silver particle formulation. But this process is very slow, which is the major drawback of this technique for silver nanoparticles formulation as compared to that of plant extracts. Thus, plants extracts are more feasible for the silver nanoparticles synthesis.

2.4.3.3 Silver Nanoparticles Synthesis by Plants

Plant extracts are easily available for silver nanoparticles synthesis and besides this, they are also safe and are non-toxic in most of the cases. They have a wide variety of metabolites which aids in the process of reduction of silver ions, therefore,

they are faster as compared to microbes. In plants associated reduction processes, phytochemicals are responsible, mainly including terpenoids, ketones, aldehydes, flavones, amides, and carboxylic acids. For immediate reduction of metallic ions, quinones, and organic acids which stand as a water-soluble phytochemicals are accountable. Several researchers have discovered that xerophytic plants contain anthraquinone and emodin which undergoes the tautomerization, and ultimately leads towards the silver nanoparticles formation. In case of mesophytic plants, researchers have found out that they have mainly three categories of benzoquinones: remirin, dietchequinone, and cyperoquinon. Thus, it was insinuated that the phytochemicals are openly intricate in the process of metallic ions reduction and also in silver nanoparticles synthesis [84].

2.5 Prerequisite for Green Synthesis

Reduction/oxidation is the main approach involved in the biosynthesis of nanoparticles. Biosynthesis gained more importance as compared to chemical and physical processes due to its cost-effectiveness. Moreover, synthesis via chemical methods is often responsible for uptake of a toxic chemical which is threatening or have hazardous effects in medical applications [85]. But in biosynthesis, uptake of toxic ions is not an issue [86]. Therefore, microbial enzymes and plant extracts (phytochemicals) are mostly used by scientists, which known antioxidant or reducing properties responsible for nanoparticles synthesis. Green synthesis is also environment-friendly, produce large-scale synthesis and doesn't require high pressure, temperature energy, and toxic chemicals.

2.5.1 Genus *Artemisia* L.

Artemisia L. belongs to family *Asteraceae* and tribe *Anthemideae*, with *Asteraceae* having more than 500 taxa [87]. It was first described by Linnaeus in his "Species Plantarum" [88]. West and Central Asia have a wide distribution of this

species and it lies in the Northern hemisphere. Whereas the southern hemisphere has very few representatives. 25 species of *Artemisia* are found in Pakistan [89]. The economic significance of this genus includes as its usage in medicines, forage, ornamentals, food and soil stabilizers [90] [91]. These species of this genus are perennial, biennial and annual herbs or small shrubs [92] [93]. *Artemisia* (Qinghao; warm wood) is also used as a traditional Chinese herb due to its high medicinal properties since 168 [94].

2.5.2 *Artemisia Carvifolia*

Artemisia carvifolia (Figure 2.3), of the genus *Artemisia*, belongs to the family *Asteraceae*. Its name was published by William Roxburgh, however, Francis Buchanan-Hamilton described *Artemisia carvifolia* first [95]. It has allelopathic (secreting chemicals) which are secreted into the ground, which inhibits the growth of other plants in its vicinity [95].The taxonomic classification is as follows:

Class: Magnoliopsida

Subclass: Asteridae

Order: Asterales

Family: Asteraceae

Genus: *A*rtemisia

Species: carvifolia

FIGURE 2.3: *Artemisia Carvifolia.*

2.6 Application of Silver Nanoparticles

Nanoparticles have an extremely small size due to which they are unique, this also refers to its large and hefty surface to volume ratio. This property leads to the physical as well as chemical differences of their characteristics and properties in comparison to the other chemicals with similar compositions such as biological, mechanical, catalytic, thermal properties and the electrical conductivity, melting point and optical absorption [96]. Therefore, variation in the size of nanoparticles by using nanometer scale can help in synthesizing novel applications, as nanoparticles exhibit properties based on size and shape. This develops the interest of researcher in developing applications i.e. computer transistors, biosensing and catalysts to optics, antimicrobial activity, chemical sensors, memory schemes,

electrometers, and wireless electronic logic. These particles as well have numerous applications in distinctive fields, for example, medical imaging, drug delivery, nano-composites, hyperthermia of tumors, and filters [97] [98].

Silver nanoparticles are mostly researched by researchers and have gained attention due to their all-encompassing functions in applications such as sensors [99], integrated circuits [22] [99], filters, bio-labeling, antimicrobial deodorant fibers [100], low-cost paper batteries (silver nano-wires) [101] antimicrobials [102] [103], and cell electrodes [101].

In the health industry, silver nanoparticles are used widely as antimicrobial agents, textile coatings, food storage and many other environmental appliances. The antimicrobial properties of silver nanoparticles are main reason for the use of nano-metals in various fields of industries, medicine, packaging, accessories, animal husbandry, military, health, and cosmetics [102] [103].

In general, remedial outcomes of silver particles (in suspension appearance) hinges upon its particle size i.e. surface area and energy. Besides this, it also depends upon the shape of the particle, its catalytic activity, the concentration of particle (therapeutic index) and charges on the particle (oligodynamic quality) [104]. The more efforts are looked-for to understand mechanisms of the antimicrobial effects of silver nanoparticles and they are yet to study in detail, but the research has shown that the silver nanoparticles attaches itself to the bacterial cell wall that is negatively charged, ruptures it, due to which protein denaturation occurs and it causes the cell death of bacteria (Table 2.2).

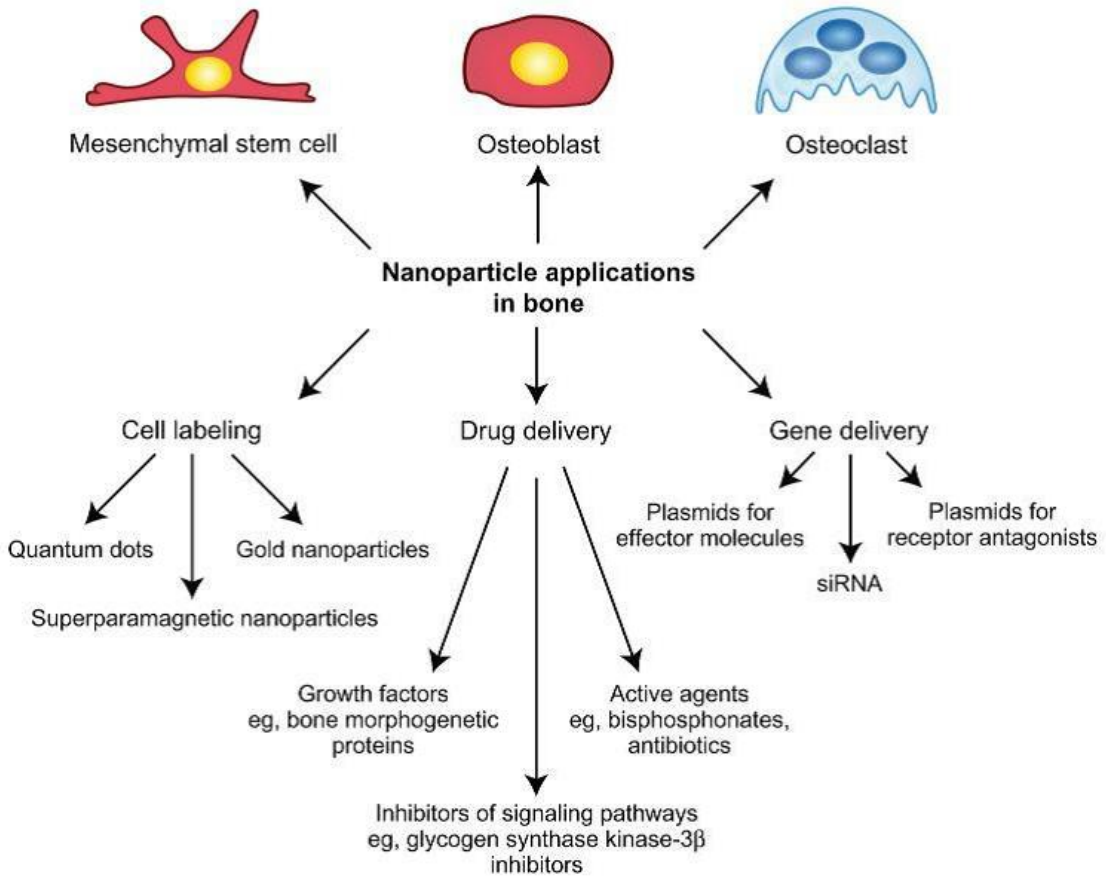

FIGURE 2.4: A general idea of applications of nanoparticles [105].

To understand, fluorescent bacterial strains were used for research on the antibacterial effects of silver nanoparticles and for this purpose green fluorescent proteins were targeted for these researches [106]. The results had uncovered that the silver nanoparticles attach itself to bacterial sulfur-containing proteins, which cause its death.

Also, the estimations of fluorescent that were without the cell supernatants have demonstrated the impact of silver nanoparticles on the bacterial recombination. This linkage of silver particles or the nanoparticles to the bacterial cell wall has triggered the amassing of precursors of envelope protein which brought about prompt dissemination of the constraining proton motive force [107].

The catalytic/synergistic mechanism of silver nanoparticle is also studied by observing composites interactions plus their damage to cell that has been caused by the compounds containing sulfur and phosphorous such as DNA [108]. Moreover,

the silver nanoparticles also flaunted the subversion of outer membrane and then ruptured the plasma membrane, thus, resulting in diminution of ATP at intracellular level [109]. Whereas an alternative mechanism includes the relationship of silver with the oxygen and its response with bunches of sulfhydryl on the groups to shape the includes the relationship of silver with oxygen and its response with sulfhydryl bunches on the groups of cell to shape R-S-S-R bonds, which blocks the respiration, thus results in the cell death (Table 2.1) [110].

TABLE 2.1: Component of antibacterial impacts on silver nanoparticles [111].

Progressions of antibacterial effects of silver nanoparticles (AgNPs)
• Induction of free radical formation leads to the cell death
• Uncoupling of oxidative phosphorylation cause the cell death
• Interference with elements of microbial ETS
• Meddling with a respiratory chain at Cyt C level
• Interactions with protein thiol groups and membrane-bound enzymes
• Collaboration with the sulfur- and phosphorous- containing compounds such as DNA

TABLE 2.2: Presentations of silver nanoparticles in dentistry, pharmaceutics, and medicine [112].

Pharmaceutics & Medicines	Molecular imaging of cancer cells Antimicrobial effects against infectious organisms Treatment of ulcerative colitis \& acne Coating of hospital textile (surgical gowns, face mask) Silver/dendrimer nanocomposite for cell labeling Detection of viral structures (SERS \& Silver nanorods) Remote laser light-induced opening of microcapsules Enhanced Raman Scattering (SERS) spectroscopy Treatment of dermatitis; inhibition of HIV-1 replication Implantable material using clay-layers with starch-stabilized Ag NPs Additive in bone cement Coating of hospital textile (surgical gowns, face mask) Orthopedic stocking Hydrogel for wound dressing
Dentistry	Silver-loaded SiO2 nanocomposite resin filler (Dental resin composite) Additive in polymerizable dental materials Patent Polyethylene tubes filled with fibrin sponge embedded with Ag NPs dispersion

Chapter 3

Materials and Methods

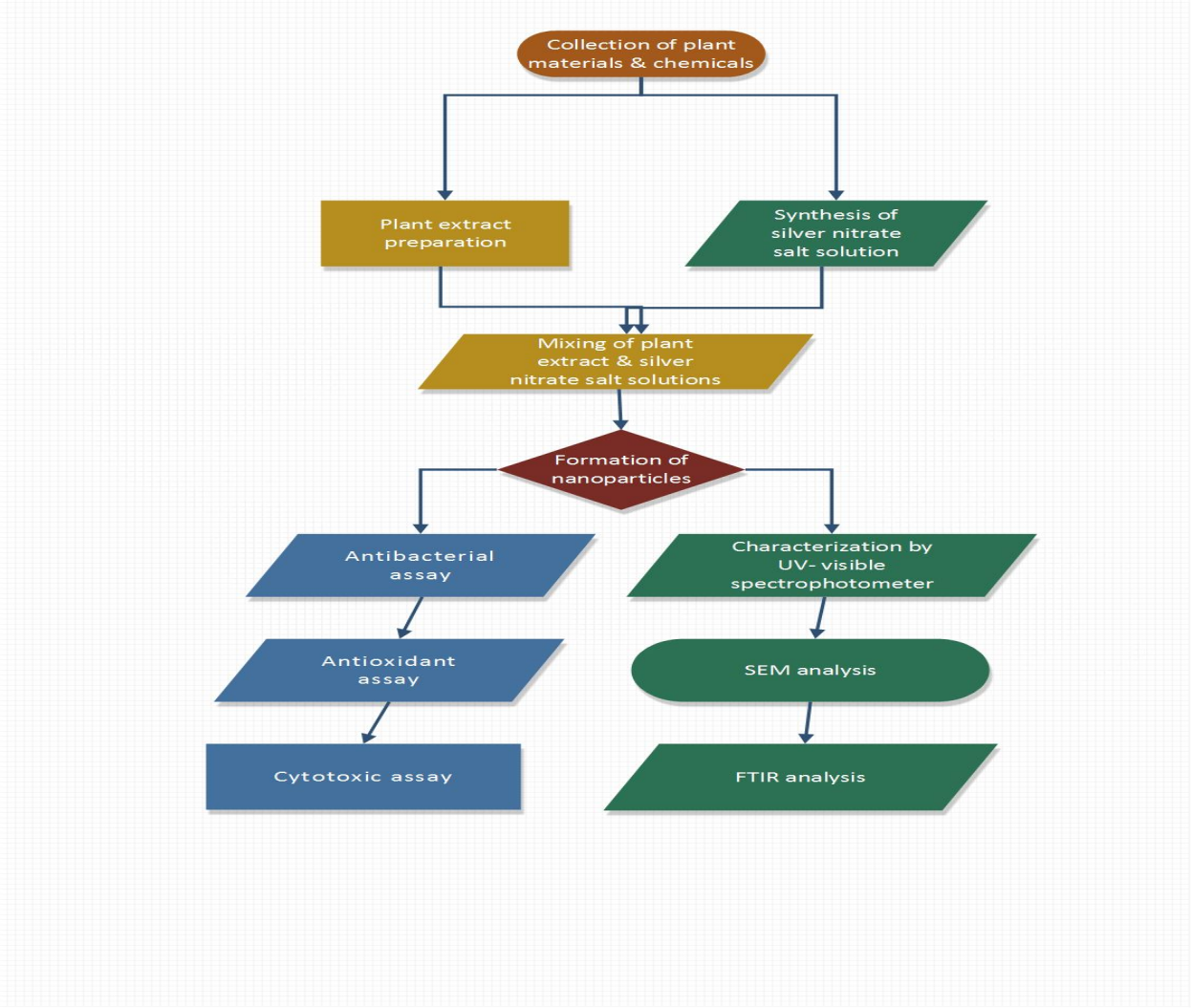

FIGURE 3.1: Overview of Methodology

3.1 Silver Nanoparticles Preparation

3.1.1 Synthesis of *Artemisia Carvifolia* Leaves Extract

Fresh leaves of *Artemisia carvifolia* (Figure 2.3), were washed numerous times with the water to remove any particles of dust or other compounds and afterward, sun-dried to evacuate the remaining moisture/dampness and then grinded/grinded to convert into powder form. At that juncture, plant extract was prepared by mixing the 160 mg of *Artemisia carvifolia* with 10 ml of distilled water in a conical flask of 250 ml and boiled for almost 10-15 minutes. Then the formed solution was filtered utilizing a Whatman filter paper. At that point, the solution was stored at 40^0C to be additionally utilized for the reduction of silver particles (Ag+) to silver nanoparticles (Ag^o)[113].

3.1.2 Silver Nitrate ($AgNO_3$) Salt Synthesis

5mM of $AgNO_3$ (Figure 3.2) was prepared by dissolving 85mg $AgNO_3$ in 100 ml of distilled water under vigorous stirring under the room temperature for about 30 min [114].

FIGURE 3.2: Silver Nitrate Salt

3.1.3 Silver Nanoparticles Synthesis

For this function, a 5 ml silver nitrate solution and 45 ml *A. carvifolia* extract were robustly mixed together in the ratio of (1:9). Then the mixture was subjected to incubation for 12 hours under sunlight. Change in the color appeared from yellow to blackish brown, after the completion of the reaction and then this bio-reduced aqueous component was subjected for the measurement of UV-Vis spectra of the synthesized solution. The obtained solution of nanoparticles were subjected to the centrifugation at the speed of 6000 rpm for about 40 minutes [16].

3.2 Silver Nanoparticles Characterization

3.2.1 Analysis of UV-Vis

The optical property of AgNPs was determined by using a UV-Vis spectrophotometer (UV-1602) (Figure 3.3). AgNPs solution was formed after the addition of $AgNO_3$ to the *A. carvifolia* extract, then 4 ml AgNPs solution were subjected to spectrophotometer by setting distilled water as a blank reference, the spectral measurements were set between 300 nm to 700 nm [113].

FIGURE 3.3: UV-Vis Spectrophotometer

3.2.2 Analysis of Scanning Electron Microscope (SEM)

Synthesized silver nanoparticles morphological features prepared from the *A. carvifolia* extract were scanned by using the Scanning Electron Microscope (JEOL-JSM-6490LATM), (Figure 3.4) operating at the voltage of 20kV (maximum) with the counting frequency of 2838 cps (maximum). Magnified micrographs were taken up to the resolution of 10 μm in scale bar. SEM slides were set up by forming a solution smear on the slides. A coat of platinum thin layer was made so that the samples become conductive [115].

FIGURE 3.4: Scanning Electron Microscope

3.2.3 Analysis of Fourier-Transform Infrared Spectroscopy (FTIR)

An FTIR spectrometer was used to study the chemical composition of the synthesized silver nanoparticles. The AgNPs solution was dried at the temperature of 75^oC and the dried powders of AgNPs were subjected to characterization in the range of 4000–400 cm^{-1} utilizing a KBr pellet strategy [116].

3.3 Biological Assessments of AgNPs

3.3.1 Antibacterial Assay

The bactericidal activity of AgNPs was concluded by using the disc diffusion method that was depicted by Ruparelia et al. [117]. Seven strains of bacteria were used for the antibacterial activities against AgNPs; four were Gram-positive, which were *Micrococcus luteus, Staphylococcus epidermidis, Bacillus subtitles,* and *Actinomyce sodontolyticus* and three Gram-negative, i.e. *Escherichia coli, Salmonella Setubal,* and *Klebsiella pneumoniae.* The positive control was antibiotic Streptomycin (100 ppm) and distilled water as negative control. $5\mu l$ of nanoparticles solution with 1000, 500, 100 and 50 ppm final concentration and *Artemisia carvifolia* with 1000 ppm concentration was poured on sterile filter paper discs and placed on nutrient agar plates. Plates were incubated at 37^oC for 24 hours. The whole procedure was carried out in triplicate. After incubation, the diameter of the zone of inhibition was measured with the help of Vernier Caliper.

3.3.2 Antioxidant Assay

The free radicals scavenging activity and total antioxidant capacity as well as total reducing power of silver nanoparticles (ACAgNPs) and *A. carvifolia* extract were evaluated using the 96 well plate (EL800 Microplate Reader) (Figure 3.5) method reported by Khalil et al. [118].

FIGURE 3.5: Scanning Electron Microscope

3.3.2.1 DPPH Free Radical Scavenging

Free radical scavenging activity was calculated by using the DPPH method [119] [120], the reagent solution (DPPH) was prepared by dissolving the 2.4mg of DPPH in ethanol. Each nanoparticle sample and plant extract sample (20μl) with a final concentration of 100 ppm, 200 ppm, 300ppm and 400 ppm was mixed with 0.1 mM DPPH (180 μl) in 96 well micro-titer plates, followed by the incubation period of 1 hour at the temperature of 37^oC. Positive control was ascorbic acid and the readings were recorded at 517 nm. The assay procedure was performed on 96 well plates (Figure 3.6). IC_{50} was calculated by using the version 4 table curve software and percent free radical scavenging was determined using formula:

$$\text{Percentage scavenging} = [(\text{control} - \text{test})/\text{control}] \times 100$$

3.3.2.2 Total Antioxidant Capacity

Phosphomolybdenum based method was used to investigate the total antioxidant capacity [121] of AgNPs and *Artemisia carvifolia*. The reagent phosphomolybdenum mixture was arranged by adding the 0.6 M H_2SO_4, $4mM(NH_4)_6Mo_7O_{24}.4H_2O$ and $28mMNaH_2PO_4$. As of the prepared test dilutions of AgNPs and *Artemisia carvifolia* extract, from them, the 20 μL were added to the phosphomolybdenum reagent solution (180 μL) that was incubated at 95^oC for about 90 minutes. At the 695 nm, readings were recorded. Results were articulated as a number of ascorbic acid equivalents (mg) per mg of the sample.

3.3.2.3 Total Reducing Power

Potassium ferricyanide ($K_3Fe(CN)_6$) based technique was used to calculate the total reducing power [122]. Concisely, 40 μL of the test samples (AgNPs and *Artemisia carvifolia* extract) were introduced to the 50 μL Phosphate-buffered saline (PBS) and afterward the reaction mixture was heated at the temperature of 50^oC for about 20 minutes in a water bath. Subsequently, to the incubation period, 50 μL of trichloroacetic acid (10%) was added in the reaction mixture of PBS and test samples. The reaction mixture after the addition of trichloroacetic acid was centrifuged for about 10 min at the speed of 3000 rpm. Conclusively, from 0.1% Ferric chloride ($FeCl_3$) 34.4 mL was added to 165.5 mL of the collected supernatant and then placed into the 96 well plate. Ascorbic acid was set as a positive control. Readings were set to be recorded at the 630 nm and the acquired results were expressed as ascorbic acid equivalents per mg of sample.

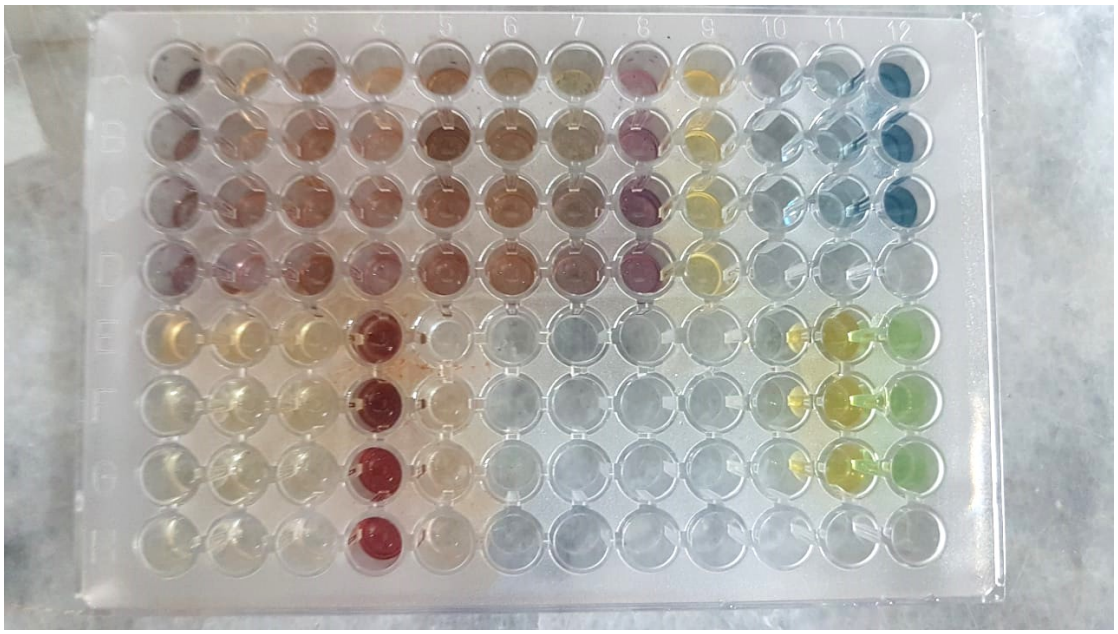

FIGURE 3.6: Test solutions in the 96 well plate

3.3.3 Cytotoxicity Assay (Brine Shrimp)

Cytotoxic assay was performed by using brine shrimps, as reported by Ismail et al. (2015), this assay was used to assess the toxicity of nanoparticles. *Artemia salina* (brine shrimp) eggs (Ocean Star Inc., USA) were hatched in seawater (34 gL1). After 24 hours shrimps were transferred to vials, almost 15 in each. The test samples of AgNPs and *Artemisia carvifolia* with final concentration of 400, 800 and 1600 μg/ml were added and final volume was made up to 5 ml. The whole procedure was carried out in triplicate. The vials were kept under light at 25^oC to 28^oC for 24 hours of incubation. After 24 hours, Surviving brine shrimp were counted by 3x magnifying glass. Percent mortality was calculated using following formula:

$$Percentagemortality = [(control - test)/control]x100$$

Chapter 4

Results and Discussions

This chapter contains the steps involved for the configuration of silver nanoparticles by using the medicinal plant extract of *Artemisia carvifolia*, and the subjection of synthesized silver nanoparticles (AgNPs) for the characterization analysis using UV-vis spectrometry, Scanning Electron Microscope (SEM), and Fourier-Transform Infrared Spectroscopy (FTIR). Silver nanoparticles were also evaluated in the biological assays that include antibacterial, antioxidant and cytotoxic activities. Results are summarized below.

4.1 Preparation of Plant Extract and Silver Nitrate ($AgNO_3$) Salt

Leaves of *Artemisia carvifolia* were dried under the sun and converted into powder form (Figure 4.1A) and these powdered leaves (160mg) were dissolved in distilled water (dH20) (10ml) and then boiled to gain the extract solution (Figure 4.1B). The resulting extracts was filtered (Figure 4.1C) and stored at 4^0C for further experiments. Afterwards the preparation of plant extract solution, the silver nitrate salt solution was synthesized by dissolving silver nitrate salt (5mM) into the distilled water (100ml) (Figure 4.2).

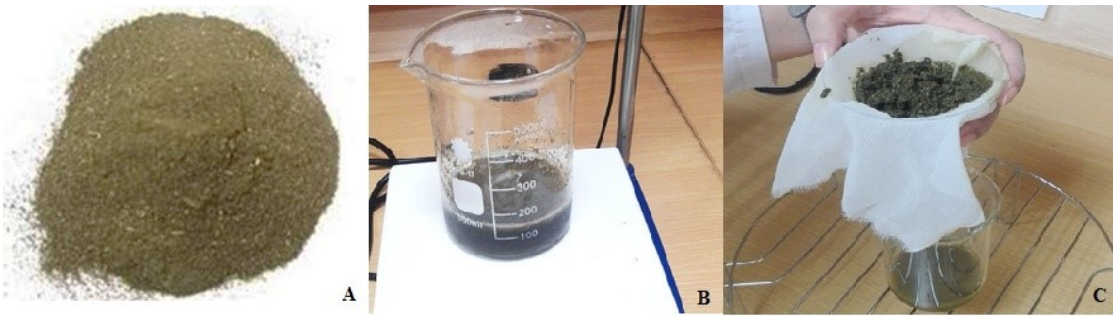

FIGURE 4.1: Synthesis of *Artemisia carvifolia* plant extract. (A) Powdered form of *Artemisia carvifolia*. (B) *Artemisia carvifolia* at boiling. (C) Filtration of *Artemisia carvifolia* plant extract.

FIGURE 4.2: Synthesis of silver nitrate salt solution.

4.2 Synthesis of Silver Nanoparticles

Subsequently the formation of solutions of *Artemisia carvifolia* plant extract and silver nitrate salt, both the solutions were mixed together in proper ratios. 45 ml of 5mM $AgNO_3$ was properly mixed with the 5 ml aqueous *Artemisia carvifolia* extract and left until color of solution was changed from yellow to blackish brown (Figure 4.3).

Gradual changes in colour from yellow to reddish yellow and from reddish yellow to dark brown were observed for aqueous solution mixture of 5mM silver nitrate.

The whole conversion of the aqueous solution from yellowish brown to blackish brown, designates the complete formation of silver nanoparticles (AgNPs). These characteristic color variations occurring are entitled to the metal nanoparticles [123].

Subsequent formation of silver nanoparticles, the prepared solution was subjected to centrifugation (Figure 4.4A). The silver nanoparticles solution was placed into the centrifugation machine and centrifuge at the maximum speed of 6000 rpm for about 40 minutes. After the pellet formation (Figure 4.4B), this pellet of silver nanoparticles was washed at least 3 times with the distilled water. The extracted pellet was dried out to form powdered silver nanoparticles in drying oven at 60^oC. After 24 hours of drying, finally, powdered silver nanoparticles were obtained (Figure 4.4C).

FIGURE 4.3: Color change during synthesis of silver nanoparticles. (A) Yellowish AgNPs of *Artemisia carvifolia* at the start of reaction. (B) Blackish Brown AgNPs of *Artemisia carvifolia* at the start of reaction.

FIGURE 4.4: Formation of powdered silver nanoparticles. (A) Centrifugation of synthesized Silver Nanoparticles. (B) AgNPs Pellet and Supernatant. (C) Powdered Silver Nanoparticles.

4.3 Characterization Analysis

4.3.1 UV-Vis Characterization

Synthesis of silver nanoparticles by the reduction of an aqueous silver nitrate into silver nanoparticles (AgNPs) during their exposure to the leaves extract of medicinal plant *Artemisia carvifolia* can be easily monitored by means of UV-visible spectrophotometer (Figure 3.3).The reduction process of Ag+ ions converting into AgNPs was confirmed using UV-Vis spectroscopy. UV-Vis spectrophotometer provides information about the size, aggregation and surface chemistry of *Artemisia carvifolia* nanoparticles. This technique provides the validation of synthesis of silver nanoparticles (AgNPs). The wavelength of the spectrophotometer was set between 300 to 700 nm. Nanoparticles contain remarkable optical properties, and these abilities are sensitive to size, shape, agglomeration state, concentration and their surface which makes spectroscopy a valuable tool for the identification and characterization of nanoparticles [124]. Silver nanoparticles interact strongly with the specific wavelength of light and optical properties [125].

Spectras of UV-Vis were collected by loading 4 mL of *Artemisia carvifolia* silver nanoparticles sample dispersion. Spectrum of spectrophotometer was background corrected by using a "blank" in which cuvette was filled with dispersing medium

(distilled water) to ensure that solvent spectral features are not included in the *Artemisia carvifolia* AgNPs sample extinction spectrum. A sharp peak increment in absorbance value was noted during the addition of silver nanoparticles solution, and then after reaching the maximum absorbance, the reaction slowed down because of the complete reduction of the Ag+ into Ag (0). A strong broad peak in the UV-Visible spectrum was observed at 450nm, while widening of spectrum peak indicates formation of AgNPs and also that the particles are poly-dispersed [126]. AgNPs are known to display an UV– Visible maximum absorption that lies in the range of 400-500nm as a result of SPR (surface plasmon resonance) (Figure 4.6) [127]. The synthesis of AgNPs (silver nanoparticles) were also optimized by consuming different concentrations of *Artemisia carvifolia* extract as 160 mg, 80 mg, 40 mg, 20 mg and 10 mg/ml. As the concentration of the *Artemisia carvifolia* AgNPs increases from 10 mg to 160 mg the absorbance intensity was increased almost about 1.5 times. This increase in the intensity of the AgNPs absorbance have also been observed by other investigators [128].The maximum absorbance was achieved using 160 mg AgNPs concentration as shown in Figure 6. Whereas the $AgNO_3$ solution and the *Artemisia carvifolia* plant extract showed no absorbance at 450 nm. These outcomes clearly demonstrate that the extent of silver nanoparticles relies upon the proportion of silver ions for their capping and stabilizing matters. Along these lines, for the development of silver nanoparticles the concentration of reducing specialists, additionally the concentrations of metal itself also retain a vital role, these results were also reported in the silver nanoparticles of *Artemisia annu*a [129]. Silver nanoparticles (AgNPs) have free electrons, which offer ascent to absorption band of surface plasmon resonance (SPR) [130], because of the joined vibration of electrons of silver nanoparticles in reverberation with the light wave [131].

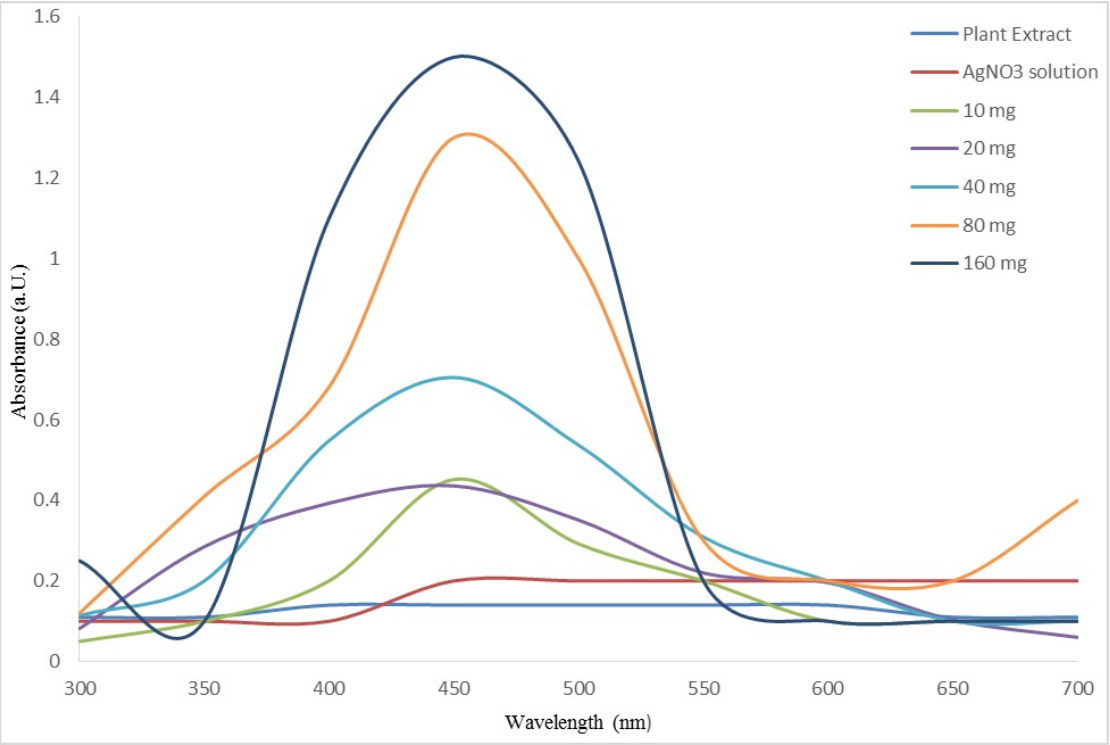

FIGURE 4.5: Spectra of *Artemisia carvifolia* plant extract, $AgNO_3$ and different concentrations of *Artemisia carvifolia* AgNPs

4.3.2 SEM Analysis of AgNPs

Further insight of size and morphology of the green synthesized silver nanoparticles were investigated using the scanning electron microscopy (SEM). The experimental outcomes exhibited that the diameters of prepared nanoparticles in the solution have the calibrated size between 80-120 nm, with average of 120 ± 06 nm. Our results have shown that the size range of the synthesized silver nanoparticles was quietly more than the usual size of nanoparticle size which ought to be; i.e.; between 1-100 nm. This size range of prepared silver nanoparticles was more than the preferred size and the reason behind this difference are the proteins which were surrounded and bounded in the surface of the nanoparticles. Similar results in which silver nanoparticles are more than the usual size was also reported by Tamasa Panigrahi [132].

The shape morphology of silver nanoparticles revealed Icosahedron (polyhedral) and leaf-shaped particles (Figure 4.6). SEM images also revealed that silver nanoparticles are much agglomerated at some points this might be due to the

magnetic behavior of silver nanoparticles and their larger surface area to volume ratio tend them to aggregate in order to reduce surface energy [133]. These particles can be coated with a biocompatible polymer to remove agglomeration [134].

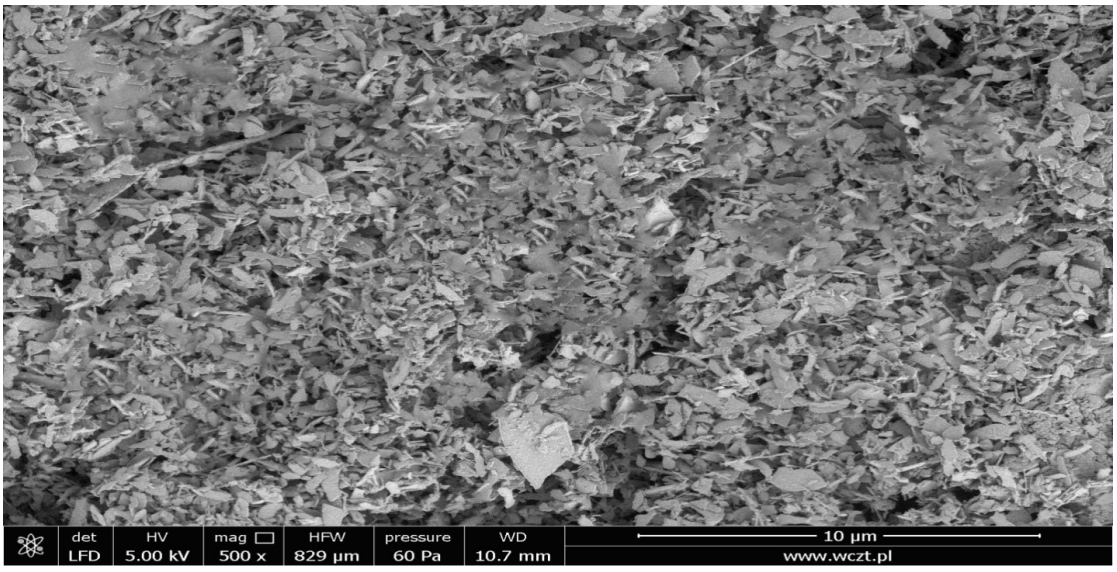

FIGURE 4.6: SEM Analysis

4.3.3 FT-IR Analysis of AgNPs

FT-IR measurements were carried out to identify the potential biomolecules in *Artemisia carvifolia* extract responsible for the reduction, capping and stabilization of the synthesized NPs. Figure 4.7 shows the spectra of FTIR of *Artemisia carvifolia* that portrays changes at position in between 800-1500 belongs majorly to aldehydes and ketones that are the major part of flavonoids, phenolics and lipid containing oils. The observed bands at the range of 1000-1140 cm^{-1} signifies the presence of the carbonyl (C–O) stretching vibration of ketones. From the investigation of FTIR examines, we affirmed that the carbonyl gatherings from the residues of amino acid and proteins has the more grounded capacity to tie metal, showing that the proteins could shape the metal nanoparticles (i.e.; capping of silver nanoparticles) to counteract agglomeration and in this manner strongly balance out and alleviate the medium. This recommends that the natural biological atoms/molecules could perform the twofold functioning for the stabilization and formation of silver nanoparticles in the aqueous medium. Similar peaks were also

reported in the AgNPs of *Artemisia marschalliana* that are representing the presence of carbonyl (C–O) stretching vibrations [134], and this significance was also reported in the AgNPs of *Cassia tora* leaf extract [135].

Correspondingly, bands between the 1100-1350 cm^{-1} may possibly belongs to the =C-O stretching of carboxyl group of lipids, which are displayed at 1308, 1309 and 1310 cm^{-1} respectively. Like wisely same medium sized peaks were also reported for carboxyl stretching in AgNPs of *Artemisia annua* [136]. There are some other band ranges at 1331-1334 cm^{-1} which experienced a change that showed the presence of carboxyl groups, thus, representing the involvement of these groups in the reduction and stabilization of the metal nanoparticle. So, from the FT-IR analysis it's been concluded that the functional groups such as C–O and =C-O groups present in the sample might be responsible for bioreduction of Ag+ to AgNPs. The observed peaks considered as a major functional groups in different chemical classes such as flavonoids, phenolics and lipid containing oils [137].

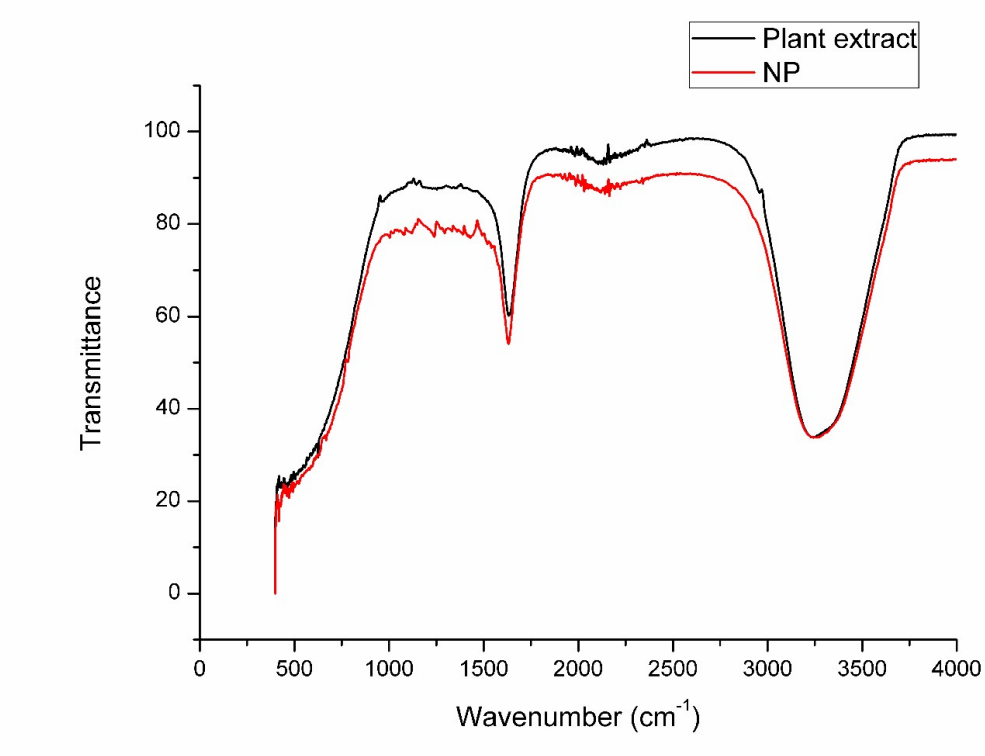

FIGURE 4.7: FT-IR spectra of *Artemisia carvifolia* plant extract and AgNPs.

4.4 Biological Assays

4.4.1 Antibacterial Activity

The antibacterial activity of synthesized *Artemisia carvifolia* AgNPs was evaluated by disc diffusion method. The results are shown in Table 4.1 and Figure 4.8, and it was observed that increasing the concentration of AgNPs progressively inhibit the growth. Seven strains of bacteria were used; four were Gram positive, which were *Micrococcus luteus, Staphylococcus epidermidis, Bacillus subtitles*, and *Actinomyces odontolyticus* and three Gram negative, i.e. *Escherichia coli, Salmonella Setubal*, and *Klebsiella pneumoniae*. Antibiotic streptomycin (100 ppm) was used as a positive control, and distilled water as a negative control. *A. carvifolia* leaf extract (1000 ppm) was also tested for antibacterial in comparison with its AgNPs. Zone of inhibition was measured using Vernier caliper.

TABLE 4.1: Results of disk diffusion assay of *A. carvifolia* AgNPs against different bacterial strains

AgNPs Concentrations (ppm)	Diameter of growth inhibition zone (mm)						
	Gram Positive Strains				Gram Negative Strains		
	M. luteus	*S. epidermidis*	*B. subtitles*	*A. odontolyticus*	*E. coli*	*S. setubal*	*K. pneumoniae*
1000	11.5	10.6	11	10	10	11	10
500	9.8	9.2	9.4	9	9	10	9.2
100	9	8.5	8.2	0	8	8.2	8.5
50	0	0	0	0	0	0	0
Plant Extract (1000 ppm)	10.1	9.9	10	9.6	9.2	9.9	9.4
Streptomycin	23	14.2	34	38	31	35	24.8

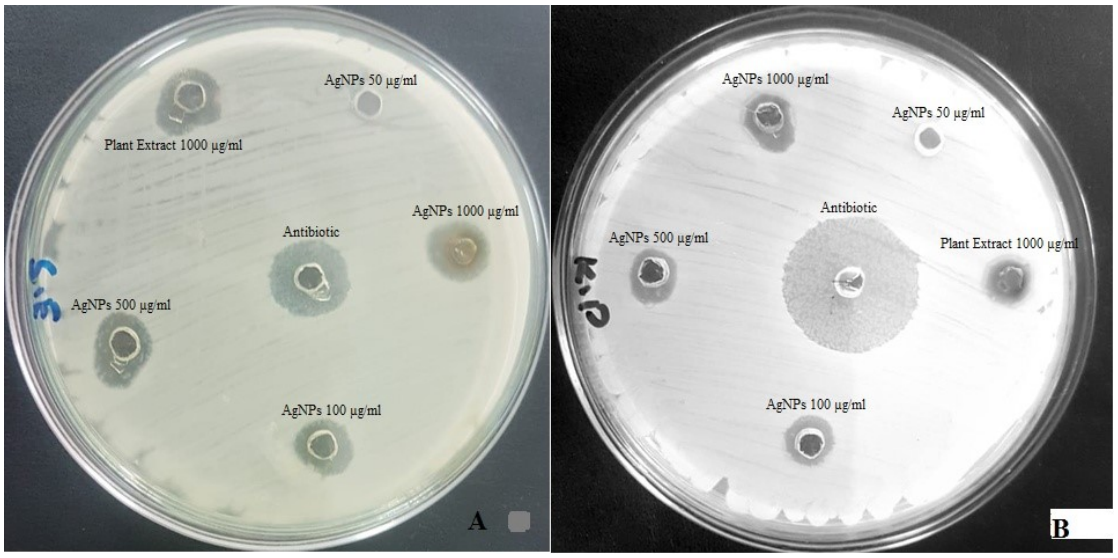

FIGURE 4.8: Antibacterial activity of synthesized *Artemisia carvifolia* Ag-NPs performed using disk diffusion method. (A)Antibacterial activity of AgNPs against gram positive strain *Staphylococcus epidermidis*. (B) Antibacterial activity of AgNPs against gram negative strain *Klebsiella pneumonia*.

To calculate the minimum inhibitory concentration (MIC) 1000ppm, 500ppm, 100ppm, and 50ppm concentration of silver nanoparticles were used. MIC for each strain was determined which is the concentration at which nanoparticles showed the least activity. For each bacterial strain including both the positive and negative, AgNPs at 1000ppm concentration showed highest zone of inhibition, whereas at the concentration of 50ppm no zone of inhibition was observed. This indicates that with the decreasing concentrations of AgNPs the activity was also decreased and ultimately at lowest concentration (50ppm) there was no activity. Also the plant extract of *Artemisia carvifolia* showed strong activity at the concentration of 1000 ppm but in comparison with the AgNPs at the 1000 ppm concentration, their activity was quite low. Our results also showed that AgNPs of *Artemisia carvifolia* exhibit strong antibacterial activity against two Gram-positive strains i.e. *Micrococcus luteus, Bacillus subtitles* and in case of Gram-negative strain, activity was shown only against *Salmonella setubal*. These green synthesized nanoparticles showed more or less same antibacterial effect against both negative and positive strains. The effect might be due to the small size of *Artemisia carvifolia* AgNPs as compared to the plant extract that enables them to penetrate into the thick walls of gram positive bacterial strains. But in literature it's been reported that

nanoparticles have more bactericidal effects for gram negative bacteria than gram positive ones due to thicker peptidoglycan cell wall [138].

AgNPs have recently received a great deal of attention and concern due to their antibacterial activity [139]. The antibacterial action of AgNPs comes about because of their infiltration into a bacterium cell, and afterward their attachment to the cell membrane surface, and then creating the disturbance in energy production by forming cell membrane damage, that is followed by the discharge of cell substance [140]. Bacterial confrontation through AgNPs is done by anchoring and then infiltrating into their cell wall, prompting an auxiliary changes in the cell membrane that results in the expansion of penetrability [141]. Mechanisms of the antibacterial activities associated with the AgNPs are attributed towards the formation of free radicals that are tempted to create the membrane damage. Silver ions interact sturdily with the thiol groups that are present in the enzymes and also in the phosphorus-containing bases, whereas the interaction of AgNPs with the DNA, may prevent the bacterial cell division and bacterial DNA replication that leads to the cell death [142]. Our results indicated that the *Artemisia carvifolia* AgNPs can be considered potential candidate for antibacterial drug, because these AgNPs are very much effective against both gram positive and negative bacteria.

4.4.2 Antioxidant Activity

Antioxidant capacities of *Artemisia carvifolia* AgNPs and *Artemisia carvifolia* plant extract was determined by using "DPPH Assay", "Total Antioxidant Capacity" and "Total Reducing Power". The biogenic AgNPs antioxidant activities are indicated in Figure 4.9. The results showed just enough and adequate activities of DPPH free radical scavenging and total reducing power, but in comparison to these, total antioxidant capacity showed a significant activity.

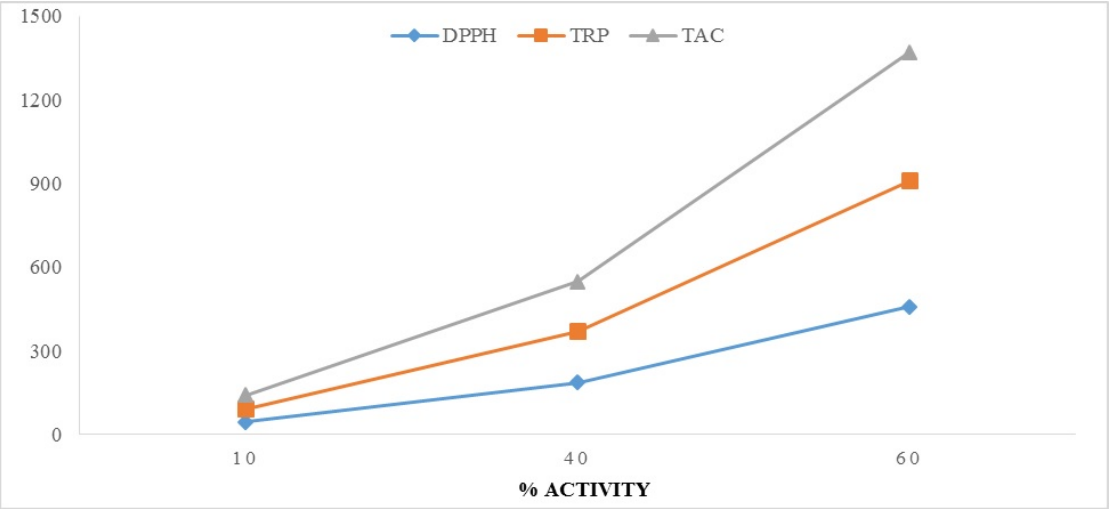

FIGURE 4.9: Comparison between DPPH assay, Total Reducing Power and Total Antioxidant Capacity of AgNPs.

4.4.3 DHHP Free Radical Scavenging

DPPH assay was performed after the 60 minutes of incubation of test samples with DPPH at room temperature. Readings were taken at the wavelength of 517 nm spectrophotometrically. Ascorbic acid, a well-known antioxidant was run as a positive control. The % scavenging activity for the AgNPs and plant extract was determined by using the % scavenging formula. The results obtained are listed in Table 4.2 which represents % scavenging of free radicals at 100 ppm, 200 ppm, 300ppm and 400 ppm and their IC_{50} values. The Percentage scavenging activities of ascorbic acid and its IC_{50} value were also calculated and are listed in Table 4.3.

TABLE 4.2: Scavenging activity and IC_{50} values of AgNPs and *Artemisia carvifolia* plant extract.

Sample Type	**Concentrations (ppm)**				
	400	300	200	100	**IC_{50}**
AgNPs	52.09%	49.57%	46.60%	45.17%	310.27
Artemisia carvifolia Plant extract	45.09%	40.48%	37.57%	34.60%	925.06

TABLE 4.3: Ascorbic acid scavenging activity and its IC_{50} value.

Positive Control	**Percentage scavenging at different concentrations**					**IC_{50} ppm**
	1ppm	3ppm	6ppm	9ppm	10ppm	
Ascorbic Acid	-78.9141	-71.0078	-25.1912	30.06406	54.55515	9.9

FIGURE 4.10: DPHH solution in Purple color.

FIGURE 4.11: Ascorbic acid turns yellow after the addition of DPHH.

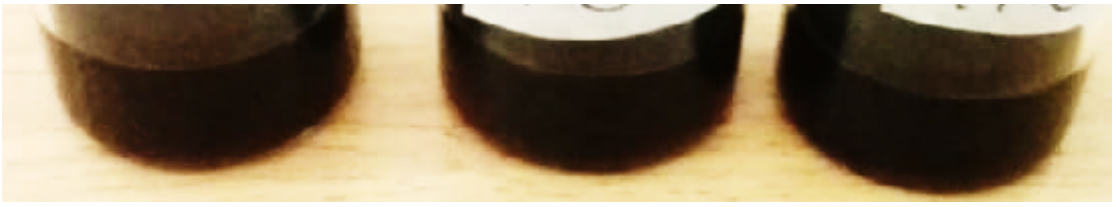

FIGURE 4.12: *Artemisia carvifolia* turns black after the addition of DPHH.

FIGURE 4.13: AgNPs turns light brown after the addition of DPHH.

DPPH is a stable free radical compound and its characteristics are to accept the electrons or hydrogen from the AgNPs. Both the AgNPs and *Artemisia carvifolia* plant extract showed significant free radical scavenging activities having the IC_{50} values 310.27 ppm and 925.06 ppm, respectively. The purple solution containing DPPH (Figure 4.10) turns black on addition of the samples of *Artemisia carvifolia* plant extract (Figure 4.12) and in case of AgNPs the solution turns into light brown as shown in Figure 4.13, which indicates the scavenging of free radicals and presence of antioxidant activity [143]. This color change due to their antioxidant activity was also reported for silver nanoparticles [144]. The free-radical scavenging results demonstrated that the levels of inhibition percentage increases with increasing concentration levels of AgNPs. The results of our investigations thus confirmed the AgNPs radical scavenging activity. *Artemisia carvifolia* plant extract and its AgNPs showed highest scavenging activity at the concentration of 1000 ppm, but in comparison, the AgNPs scavenging activity is higher than the *Artemisia carvifolia* extract as shown in Table 1.The potential explanation behind the antioxidant activity of AgNPs might be associated to the presence of bioactive compounds exhibited in them. Nanoparticles are known to exhibit scavenging activities and enhanced DPPH scavenging properties of *Artemisia marschalliana* silver nanoparticles were also reported [134].

4.4.4 Total Antioxidant Capacity

The total antioxidant activity of the *Artemisia carvifolia* plant extract and its AgNPs was evaluated (Table 4.4) by using the phosphomolybdenum method. This assay was performed by using the 96 well plate (Figure 3.6) after the 90 minutes of incubation of test samples at 95^oC. Readings were taken at the 695 nm. Ascorbic acid was run as a positive control. It is a quantitative method to investigate the reduction reaction rate of antioxidants [145]. It involves the thermal generation of an auto-oxidant through extended incubation at higher temperatures [146]. It illustrates straight estimation of the reducing capacity of the antioxidant [147]. Well according to the results obtained from antioxidant capacity assays, all the test

samples including AgNPs at the value of 457.00 3.57, *Artemisia carvifolia* extract at the value of 447.11 3.57 and ascorbic acid (positive control) readings at 111.51 0.05, showed moderate antioxidant activity but the *Artemisia carvifolia* silver nanoparticles has shown a significant total antioxidant capacity as compared to the extract as shown in Figure (4.14). This comparison between the plant extract and AgNPs clearly indicates that the AgNPs has strong and more effective antioxidant potential as compared to the extract of *Artemisia carvifolia*. Therefore, these AgNPs can be perfectly used for scavenging activities and even preferably over the medicinal plants because these antioxidants agents are useful in protecting cells from oxidative damage [148].

TABLE 4.4: Total Antioxidant Activity of AgNPs, *A. carvifolia* extract and ascorbic acid.

S.no	Test Samples	Mean ± Standard Deviation
1	AgNPs	457.00 ± 3.57
2	*Artemisia carvifolia* Extract	447.11 ± 3.57
3	Ascorbic Acid	111.51 ± 0.05

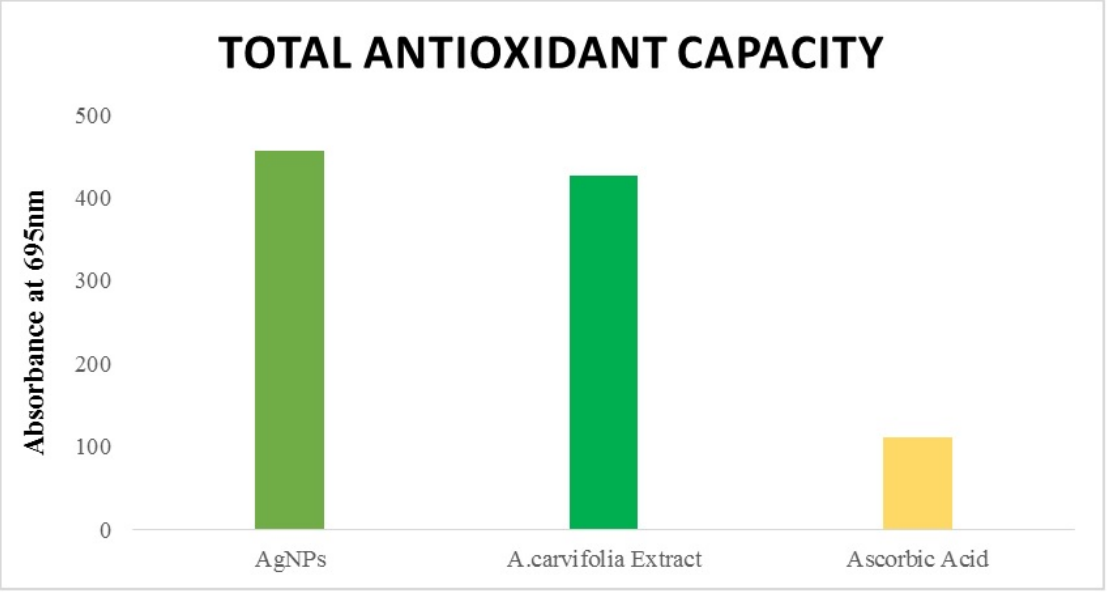

FIGURE 4.14: Graph of Total Antioxidant Capacity of AgNPs, *A carvifolia* extract and ascorbic acid.

4.4.5 Total Reducing Power assay

Potassium ferricyanide ($K_3Fe(CN)_6$) based method was used to calculate the total reducing power. The electron donating capacity (reducing power) of compound is associated with antioxidant activity [149]. All the test samples including AgNPs at the value of 186.09±3.76, *Artemisia carvifolia* extract at the value of 175.10±3.11 and ascorbic acid (positive control) readings at 158.20 ± 0.05 (Table 4.5), showed a significant reducing power, but the reducing power of silver nanoparticles is higher as compared to *Artemisia carvifolia* extract as shown in Figure 4.15. Silver nanoparticles have shown more reducing power than standard. Reducing power is evaluated by the transformation of Fe_3+ to Fe_2+ in presence of compound [150]. The reducing capacity of silver nanoparticles may serve as significant indicator of its potential antioxidant activity [151]. This comparison between the plant extract and AgNPs clearly indicates that the AgNPs has strong and more effective reducing potential as compared to the extract of *Artemisia carvifolia*.

TABLE 4.5: Total Reducing Capacity of AgNPs, *A. carvifolia* extract and ascorbic acid.

S.no	Test Samples	Mean ± Standard Deviation
1	AgNPs	186.09 ± 3.76
2	*Artemisia carvifolia* Extract	175.10 ± 3.11
3	Ascorbic Acid	158.20 ± 0.05

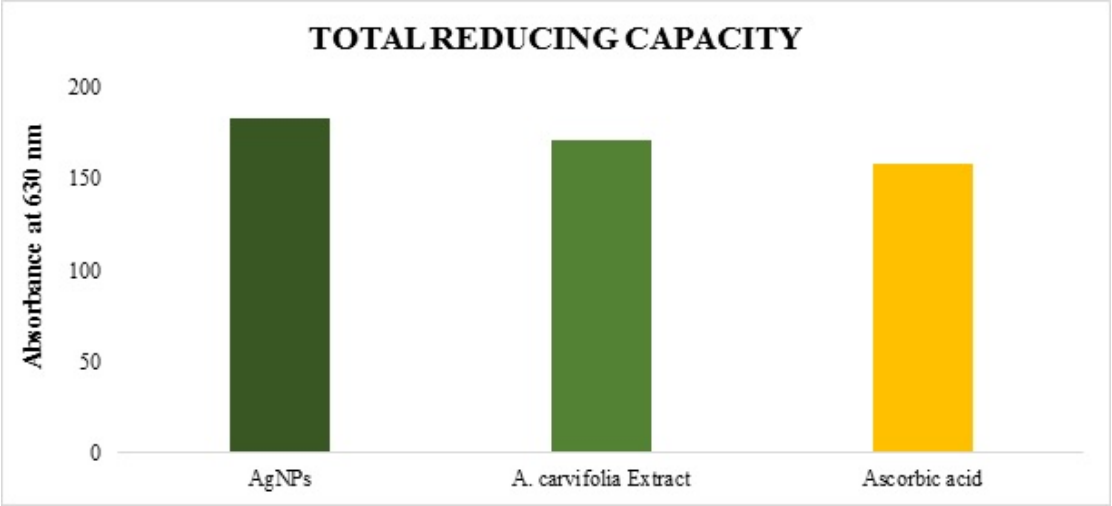

FIGURE 4.15: Graph of Total Reducing Capacity of AgNPs, *A. carvifolia* extract and ascorbic acid.

4.4.6 Cytotoxic Assay

To evaluate toxic behavior, silver nanoparticles were subjected to brine shrimp cytotoxic assay. Cytotoxic brine shrimp performances of any plant extract determine their different pharmacological properties [152]. The results indicated the toxic effect of silver nanoparticles with 100% mortality. In this study, three different concentrations; 400, 800, and 1600 μg/ml of silver nanoparticles and extract of *Artemisia carvifolia* were exhausted to acquire their cytotoxicity levels by means of the brine shrimp lethality assay. Higher concentrations had more mortality rate than lower concentrations of silver nanoparticle. The LD_{50} value of silver nanoparticles were at the 19.06 μg/ml, whereas the *Artemisia carvifolia* extract LD_{50} was hit upon up at the 87.13 μg/ml (Table 4.6). The lowered LD_{50} value advocated that cytotoxic nature of the synthesized AgNPs are quite excessive as compared to the extract of medicinal plant. This enhanced and enriched cytotoxicity of the silver nanoparticles having LD_{50} of 19.06 μg/ml to brine shrimp lethality assay revealed the existence of toxic components. Ag ions mainly contribute to cytotoxic and stress associated effects. Nano-size alone has unique toxic effects on the cells, which suggests that both particles and dissolved ions can synergically influence cellular responses [153].Cytotoxic effects of silver nanoparticles towards the larvae of brine shrimp can be associated with the anti-cancerous activity and these metallic silver nanoparticles have the potential to be utilized as an alternative source of anticancer drugs [114].

TABLE 4.6: Cytotoxic Activity of AgNPs and *A. carvifolia* Extract.

S.no	Test Samples	Percentage of brine shrimps killed after 24 h			LD_{50} µg/ml
		400 µg/ml	800 µg/ml	1600 µg/ml	
1	AgNPs	85.71%	92.30%	97.46%	19.06
2	*Artemisia carvifolia*	84.82%	86.41%	93.57%	87.13

Chapter 5

Conclusion and Future Work

The fast and swift natural biological synthesis of silver nanoparticles utilizing *Artemisia carvifolia* medicinal plant provides ecological well disposed, friendly, simple, straightforward, and effective routes for the synthesis of considerate nanoparticles. The synthesized silver nanoparticles in this study were of polyhedral as well as leaf-shaped and the evaluated sizes of these nanoparticles were between the 80 -120 nm. This estimated size was greater because these silver nanoparticles were incorporated by a thin layer of metabolites and proteins, for example, alcohols, amines, aldehydes, ketones, and so forth, which were discovered from the descriptive characterization by utilizing the UV-vis spectrophotometer, SEM, and FTIR strategies. Each one of these techniques has exhibited that the proportion of medicinal plant extract concentrations to metal ions play a basic part in the shape affirmation of the nanoparticles. Whereas the nanoparticles of higher concentration had a leaf-shaped appearance. The sizes of the nanoparticles in various concentrations were correspondingly dissimilar which rely upon the diminishment of metal ions. Both the silver nanoparticles and *Artemisia carvifolia* medicinal plant proved to be good antibacterial agents against gram +ve as well as gram -ve bacteria, but in comparison with the medicinal plant, silver nanoparticles had strong activity. Among all antioxidant assays, synthesized silver nanoparticles had shown a strong antioxidant potential as standing against a known antioxidant medicinal plant. Astonishingly silver nanoparticles have also displayed a

strong powerful cytotoxic potential. The biological activity of synthesized silver nanoparticles was more than their corresponding salts and extracts. So these stable and toxic metallic nanoparticles can be used in targeted drug delivery and treatment of various diseases. From a technological perspective, these acquired silver nanoparticles have a potential applications in the biomedical field and this straightforward system has a few points of interest, for example, cost-adequacy, correspondence for therapeutic and pharmaceutical applications and in addition huge scale commercial productions.

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